

FIG. 1A

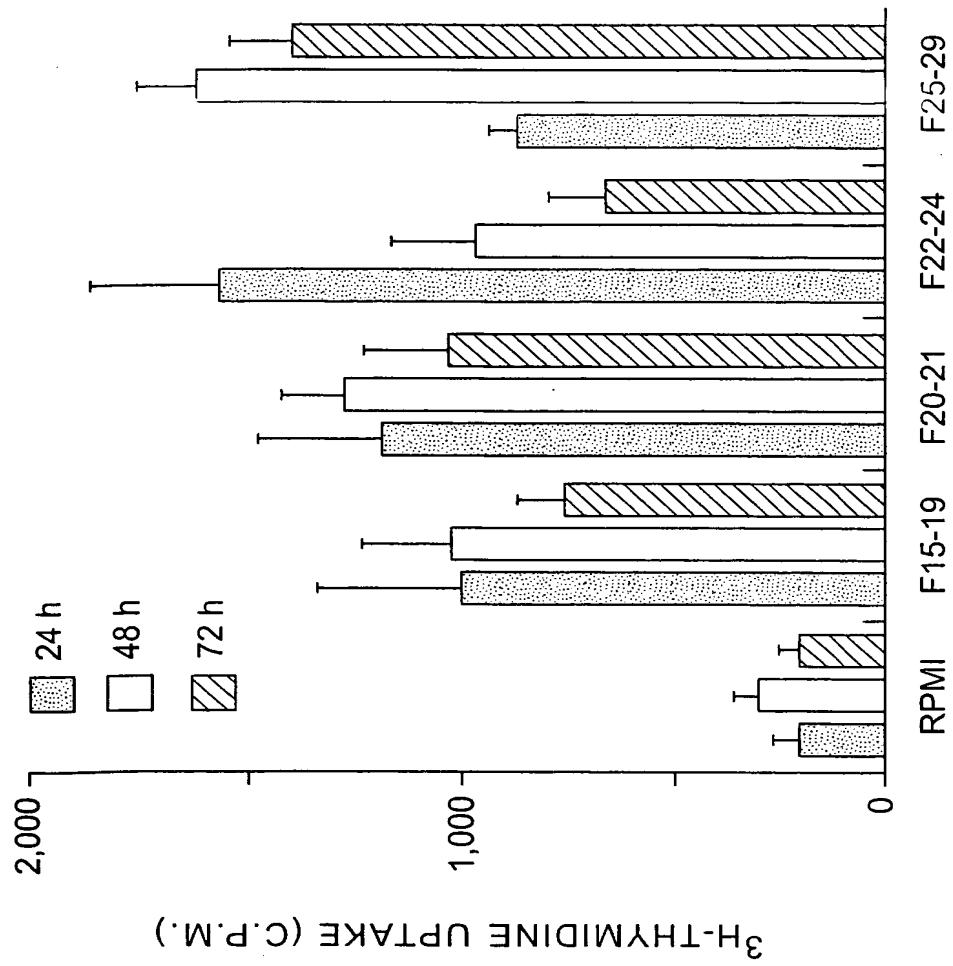


FIG. 1B

HPLC FRACTIONS

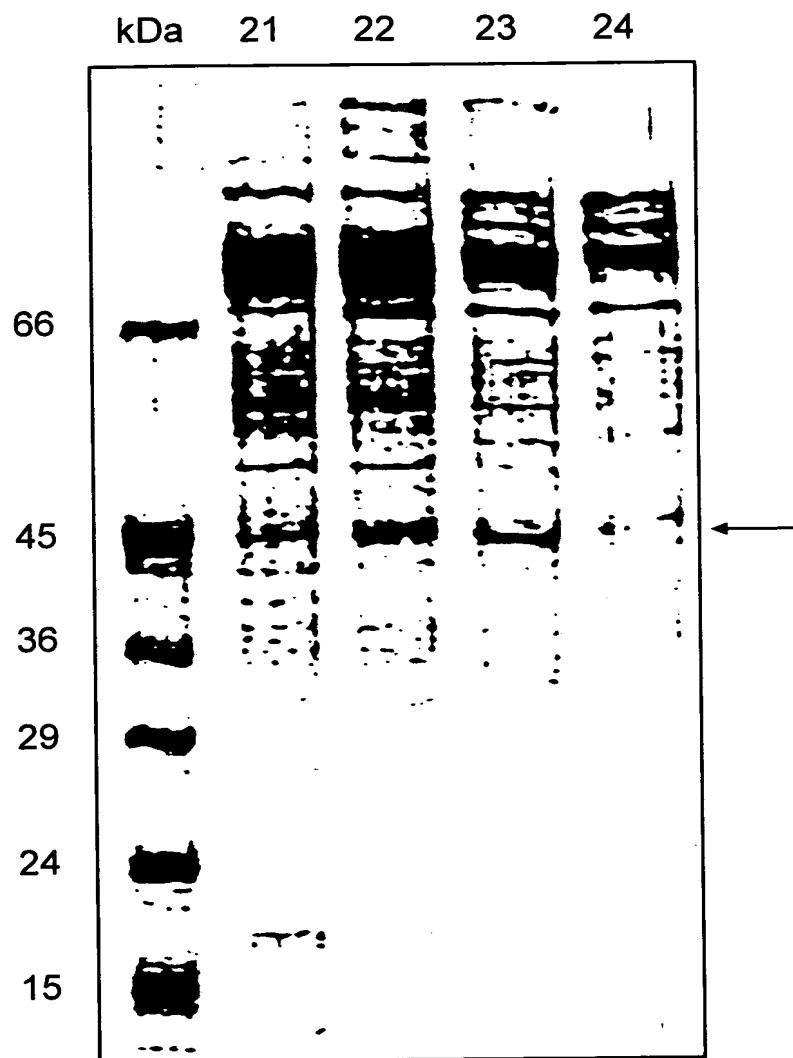


FIG. 1C

TC	MRKSVCPKQKFFFSAFPFFFCVFPLISRTGQEKLFDQKYKIIKGEEKKKNQANRREHQOKREIMREKK	75	6
CS	-----	-----	MKF SKG
Pa	-----	-----	MQR
TC	FTC1DMHTEGEAARIVTSGLPHIPGSNMAEKKA YLQENMDYLRRGIMLEPRGHDDMFGAFLFDPIEGADLGMVF	150	81
CS	IHAIDSHTMGEPTTRIVVGGIPOINGETMADKKKYLEDNDVYVRTALMHEPRGHNDMFGSIITSSNNKEADFGIIF	151	78
Pa	I RIIDSHTGGEPTTRLVIGGFPDLGQDMAERRRLIGERHDAWRAACILEPRGSDVLLVAGALLCAPVDP	140	
TC	MDTGGYLMCGHNSIAAVTAAVETGIVSVPAKATNVPVVLDTPAGLVRGTAHLQSGTSESEVSNASIIINVPSFLYQ	225	
CS	MDGGGYLMCGHNSIGAATVAETGMVEMVEPTVNIN--MEA PAGL IKA KV MVEN--EVKVEVSITNVPSFLY	151	
Pa	FNNSGYLGMC GHGTIGLVASLIAHGRIGPGV--HRIETPVGEVEATLH-----EDGSVSVRNVPAYR	208	
TC	QDVVVVLBPKPYGEVRVDIAFGGNFFAIVPAEQLGIDISVONLSRLOEAGELLRTEINRSVVKVQHPQLPHINTVDC	300	
CS	EDAKLEVPSLNKTITFDISFGSSFFAIIAKELJGVKVE TSQVDVLKKLGIEIRDLINEKIKVQHPELEHIKTV	226	
Pa	RQSVSEVPGI-GRVSGDIAWGNWEFLVAGH--GQRLAGDNLDALTAYTVAQQALDD----QDIRGEDGAI DH	208	
TC	VEIYGPPTNPEANYKNVVIFGNRQADR	371	
CS	VEIYDEPSNPEATYKNVVIFGQQQVDR	297	
Pa	I ELFAD--DPHADSRNFVILCPGKAYDR	279	
TC	SPCGT GTS A KMATLYAKGQLRIGETFVYESILGSLI FQGRV--LGEE	423	
CS	SPCGT GTS A KLA TLYKGKGHLKIDEKFVYESITGTMFKGRV--LEET	335	
Pa	SPCGT GTS A KLA CLA ADGKL P GQP W RQ AS VIGSQ FEGRYEWLDGQ	314	
TC	RIPGVKVPVTKAEEGMLVVTAEITGKAFIMGNTMLFDPTDFKNGFTLKQ*	423	
CS	KVGEFD-----AIPEITGAYITGFNHFVIDPEDPLKYGFTV*	335	
Pa	PGGPIVPTIRGRAHVSAEATLLLADDDPFAWGIRR*	314	

FIG. 2

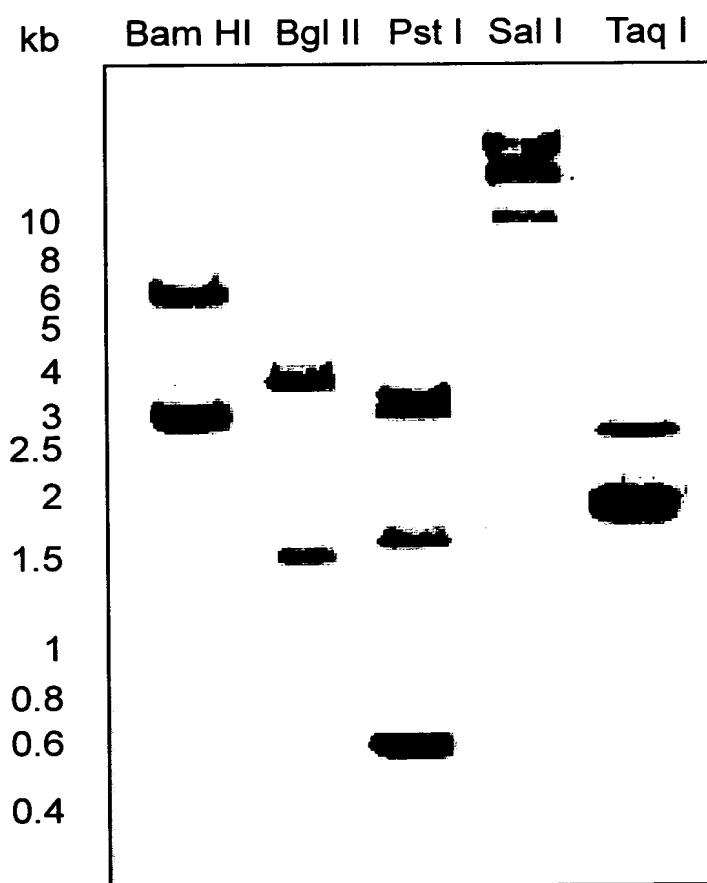


FIG. 3A

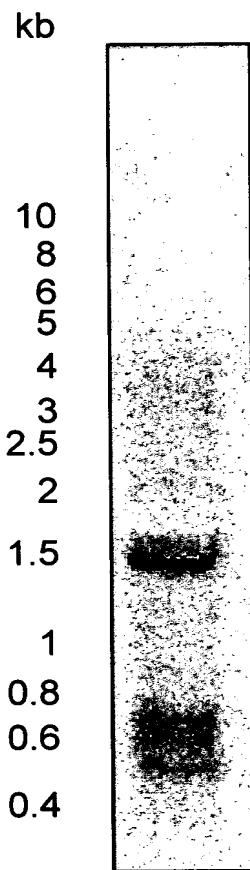


FIG. 3B

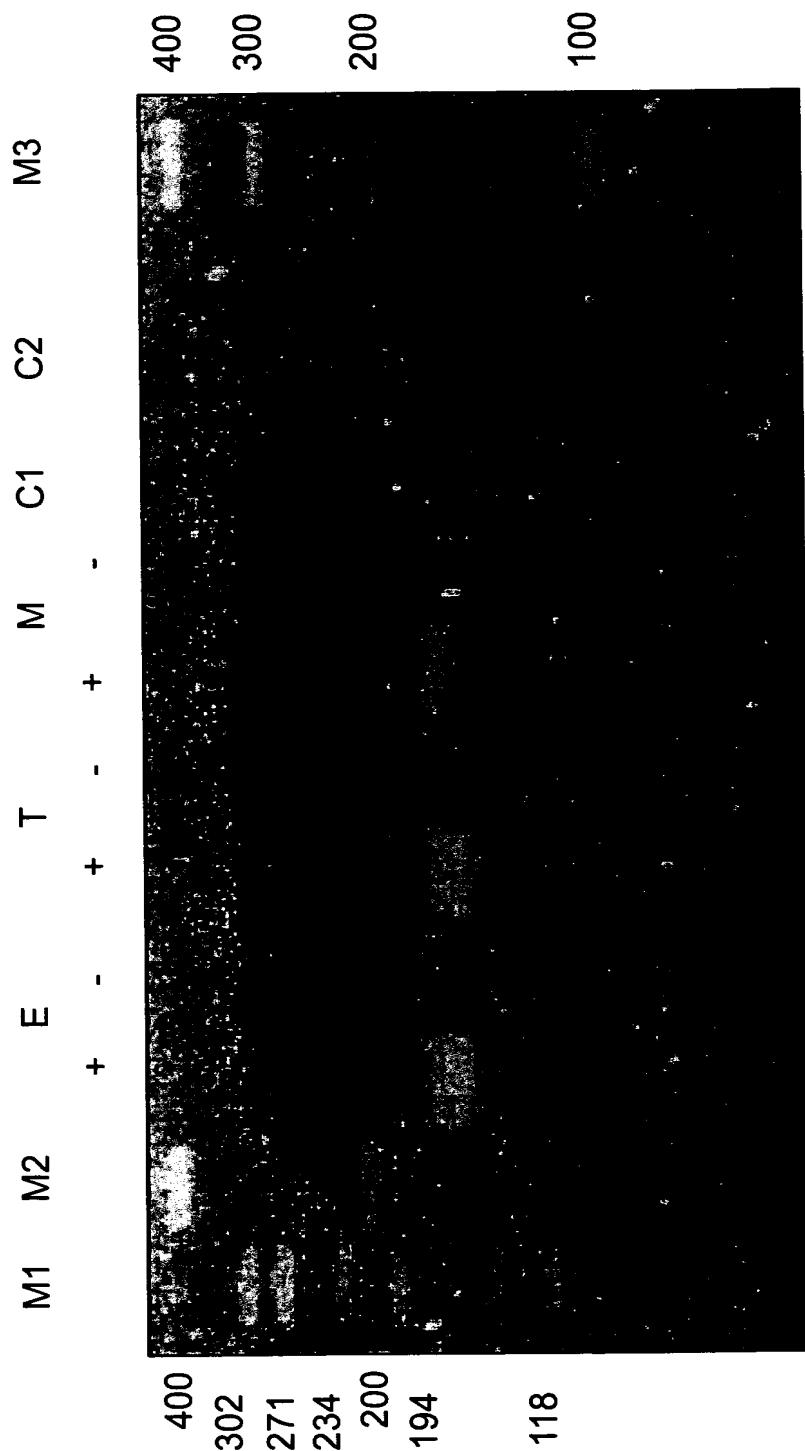


FIG. 3C

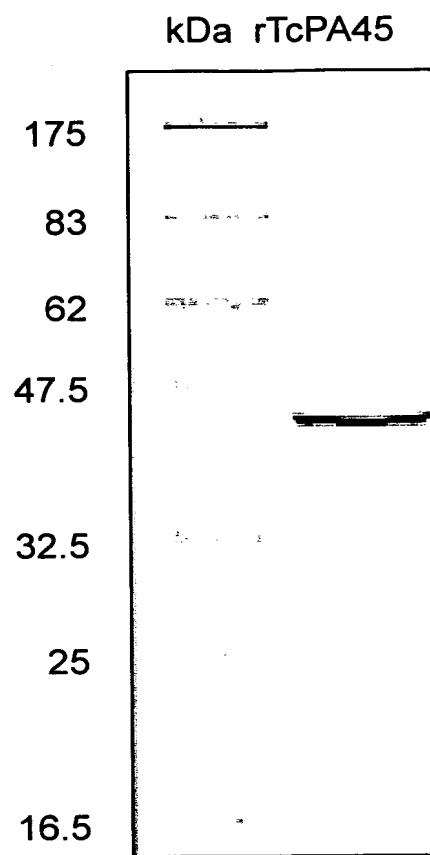


FIG. 4A

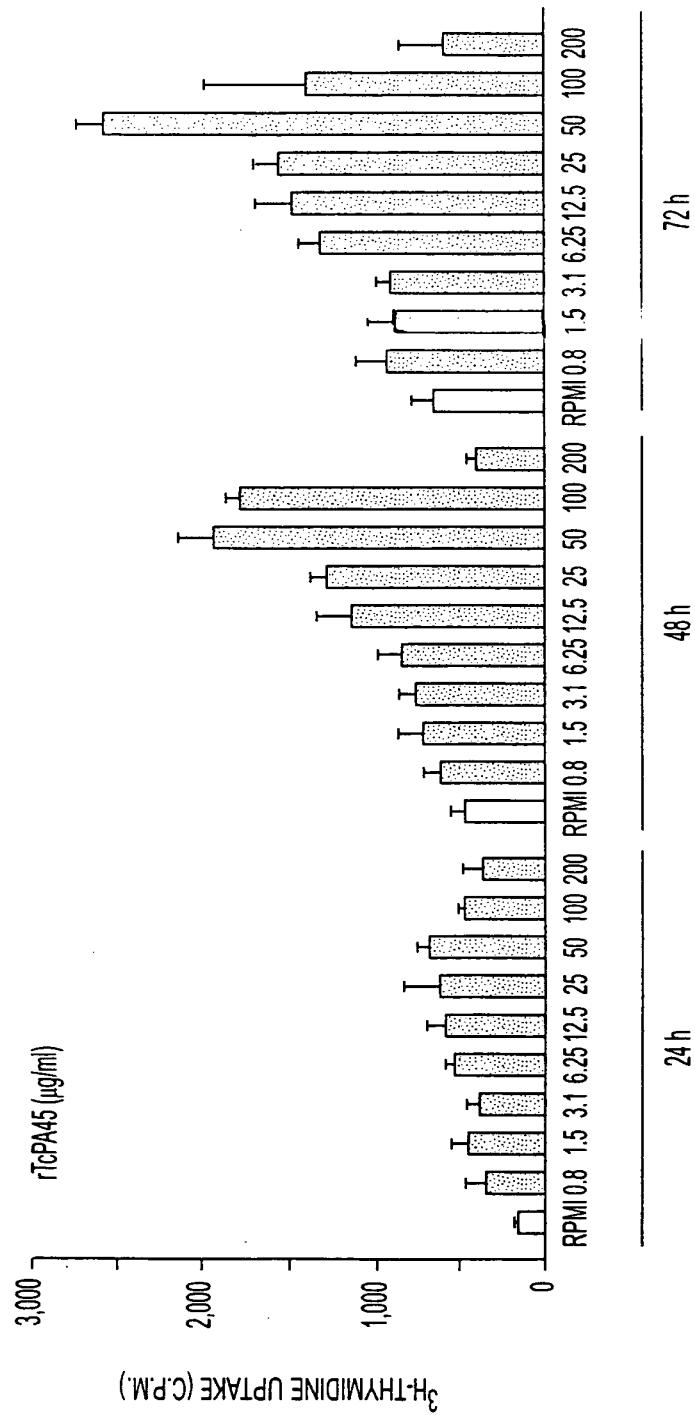


FIG. 4B

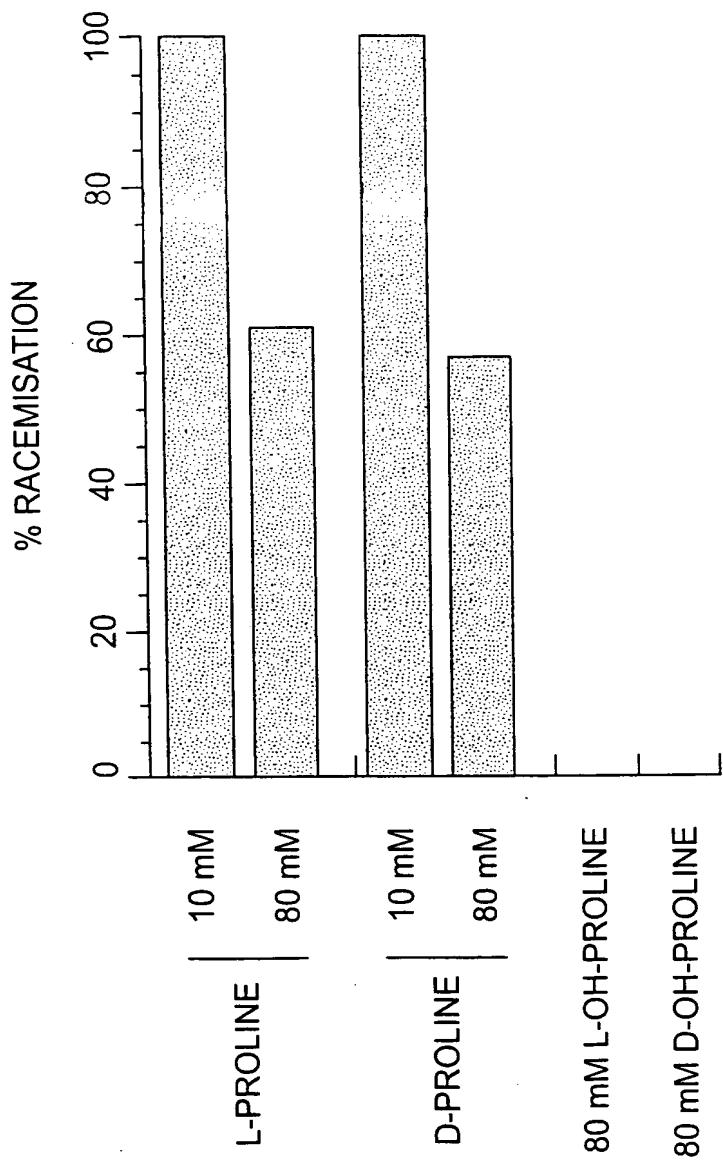


FIG. 4C

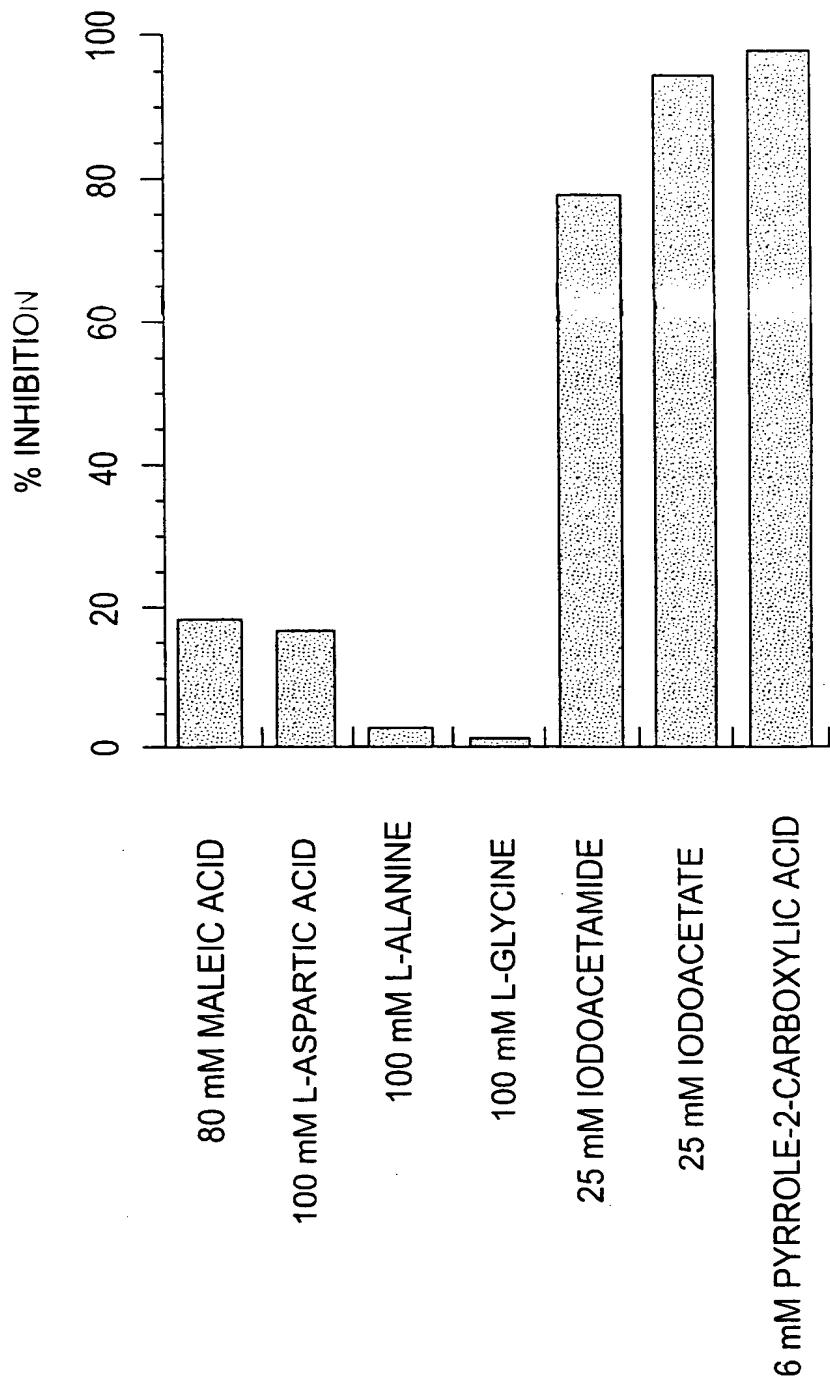
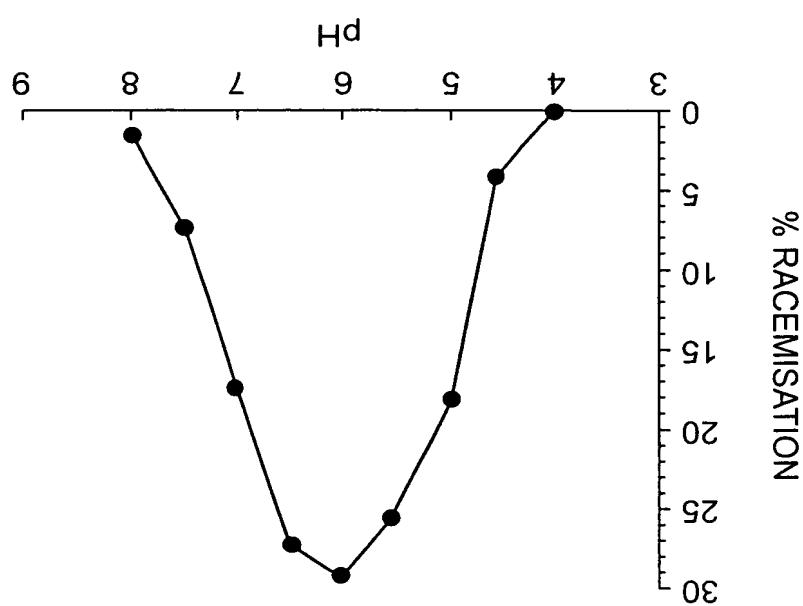


FIG. 4D

FIG. 4E



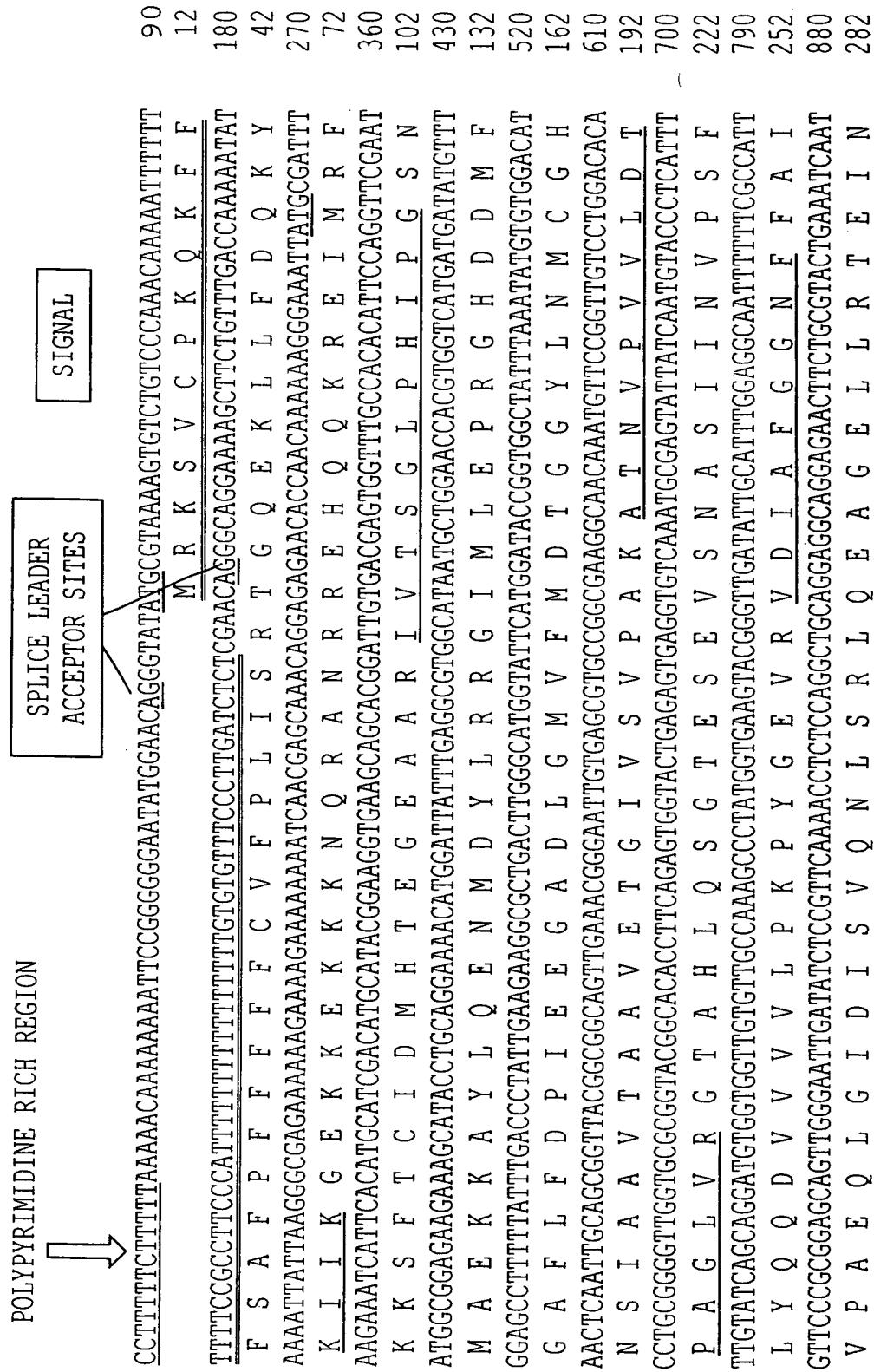


FIG. 5A

CGCAGTGTGAAGGTTCAAGCACCCCTCAGGTGCCCATATAACACTGTGCACTGTGTGAGATATAACGGTCGCCAACGAACCCGGAGGCA 970
R S V K V Q H P Q L P H I N T V D C V E I Y G P P T N P E A 312
AACTACAAGAACGTTGTGATATTGGCAATGCCAGGGATCGCTCTCCATGTGGCACAGGCCACCGCCAAAGATGGCAACACTTTAT 1060
N Y K N V V I F G N R Q A D R S P C G T G I S A K M A T L Y 342
GCCAAAGGCCAGCTTCGCATGGAGACTTTGTGTACGGAGGCTACTCGGCTCAACTCTCAGGGCAGGGTACTTGGGGAGGCCA 1150
A K G Q L R I G E T F V Y E S I L G S L F Q G R V L G E E R 372
ATACCGGGGTGAAGGTGCCGGTACCAAGATGCCAGGAAGGGATGCCAGGGATGCCGTGTAAACGGCAAATTACTGGAAAGGCTTTATCATG 1240
I P G V K V P V T K D A E E G M L V V T A E I T G K A F I M 402
GGTTTCAACACCATGCTGTTGACCCAACGGATCCGGTTAACAGAACGGATTCACATTAAAGCAGTAGATCTGGTAGAGCACAGAACTATT 1330
G F N T M L F D P T D P F K N G F T L K Q 423
GGGAAACACGTGCCAACAGGTGCTACGTGAAGGGTATTGAATGAATCGTTTTTATTATTATTATTAGTCATT 1420
ATTATTAATTTTTTTGGGGTTCAACGGTACCGCTGGAGCAGGAAGGGATAGGGGGGACAAATTTCGGTTTAT 1510
TTTCATTTCATCTTCTACCCAAACCCCTGGTTCCACCGTCCGGGGGTCTGTGGGGAGGAGTCCTAAATCCGGACCTCGG 1600
AGGAATAAACATATTTCATATCTTGGAAATCAAAGGCAT 1651

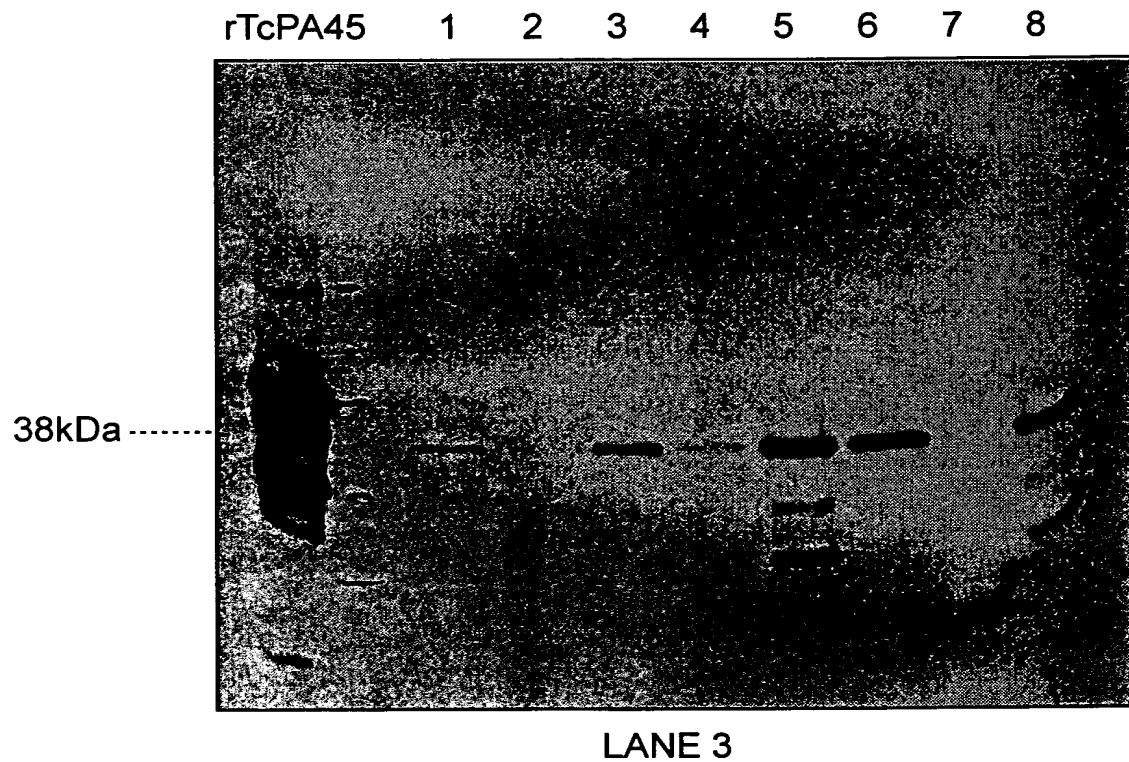
POLYADENYLATION SITE

OBS: UNDERLINED THE SEQUENCED PEPTIDES USED TO DEDUCE DEGENERATED PRIMERS FOR CLONING

NUCLEOTIDE SEQUENCE AND PEPTIDE SEQUENCE TCPA45

FIG. 5B

WESTERN BLOTTING



SOLUBLE FRACTION OF EPIMASTIGOTES EXTRACT (CYTOSOLIC)
REVEALED WITH ANTIBODY DIRECTED TO rTcPA45

----- DEMONSTRATES THE EXISTANCE OF AN INTRACYTOPLASMIC
FORM OF TcPA45 IN THE PARASITE

FIG. 6

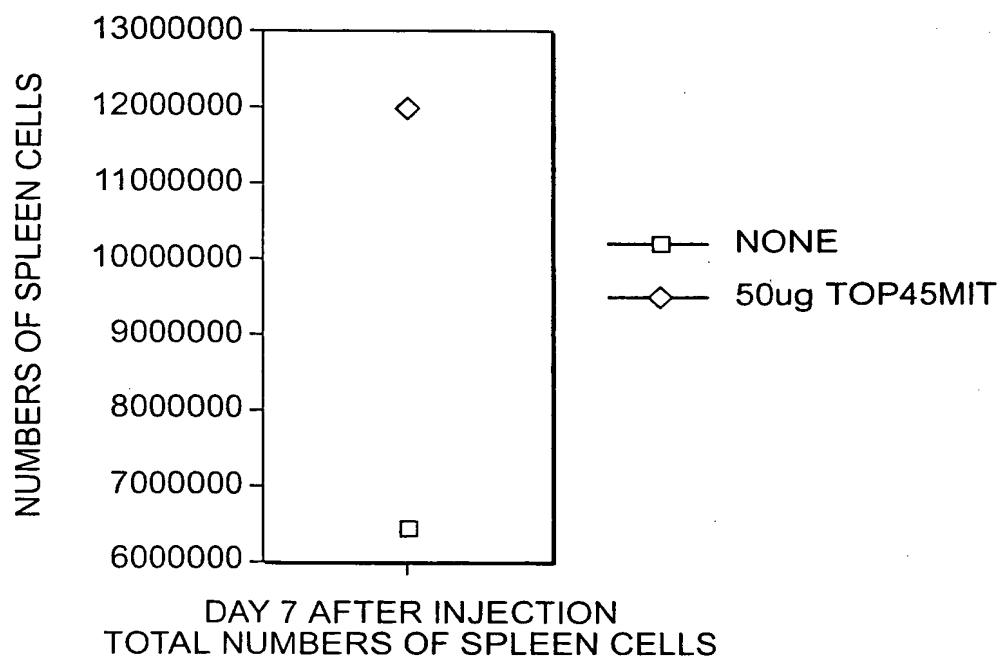


FIG. 7

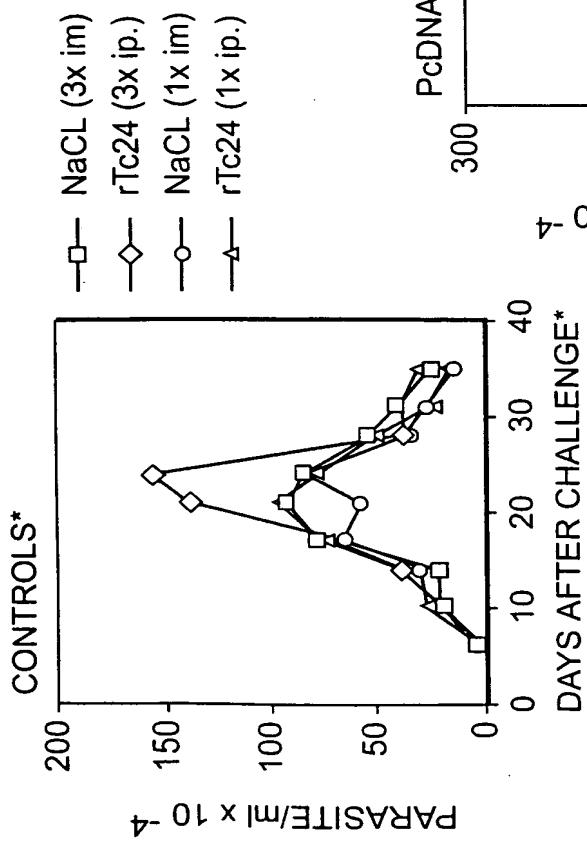


FIG. 8A

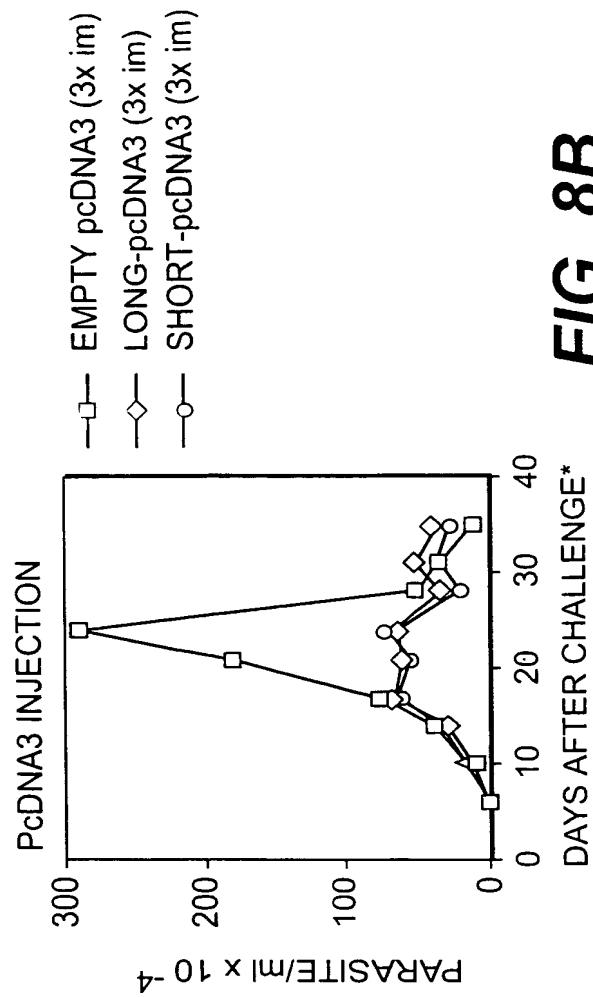


FIG. 8B

—□— EMPTY pcDNA3 (1x i.m.) —□— EMPTY VR 1020 (3 i.m.)
—◇— LONG pcDNA3 (1x i.m.) —◇— LONG VR 1020 (3 i.m.)
—○— SHORT pcDNA3 (1x i.m.) —○— SHORT VR 1020 (3 i.m.)

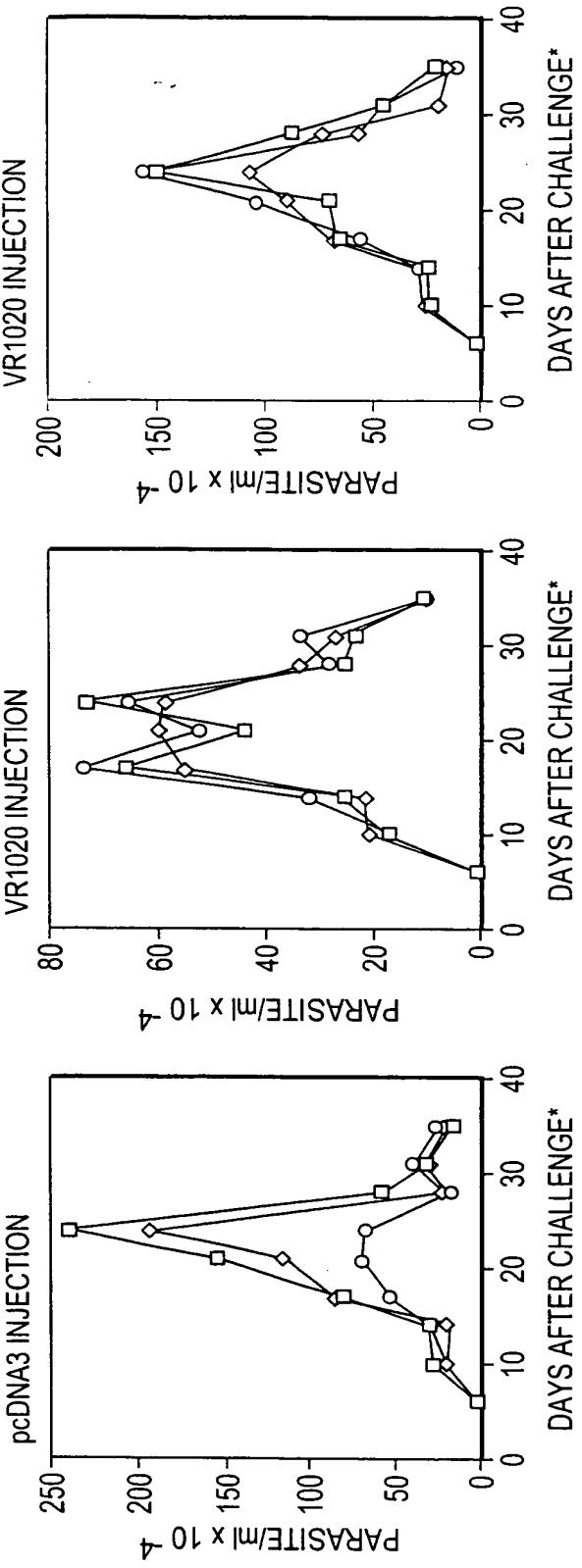


FIG. 8C

FIG. 8D

FIG. 8E

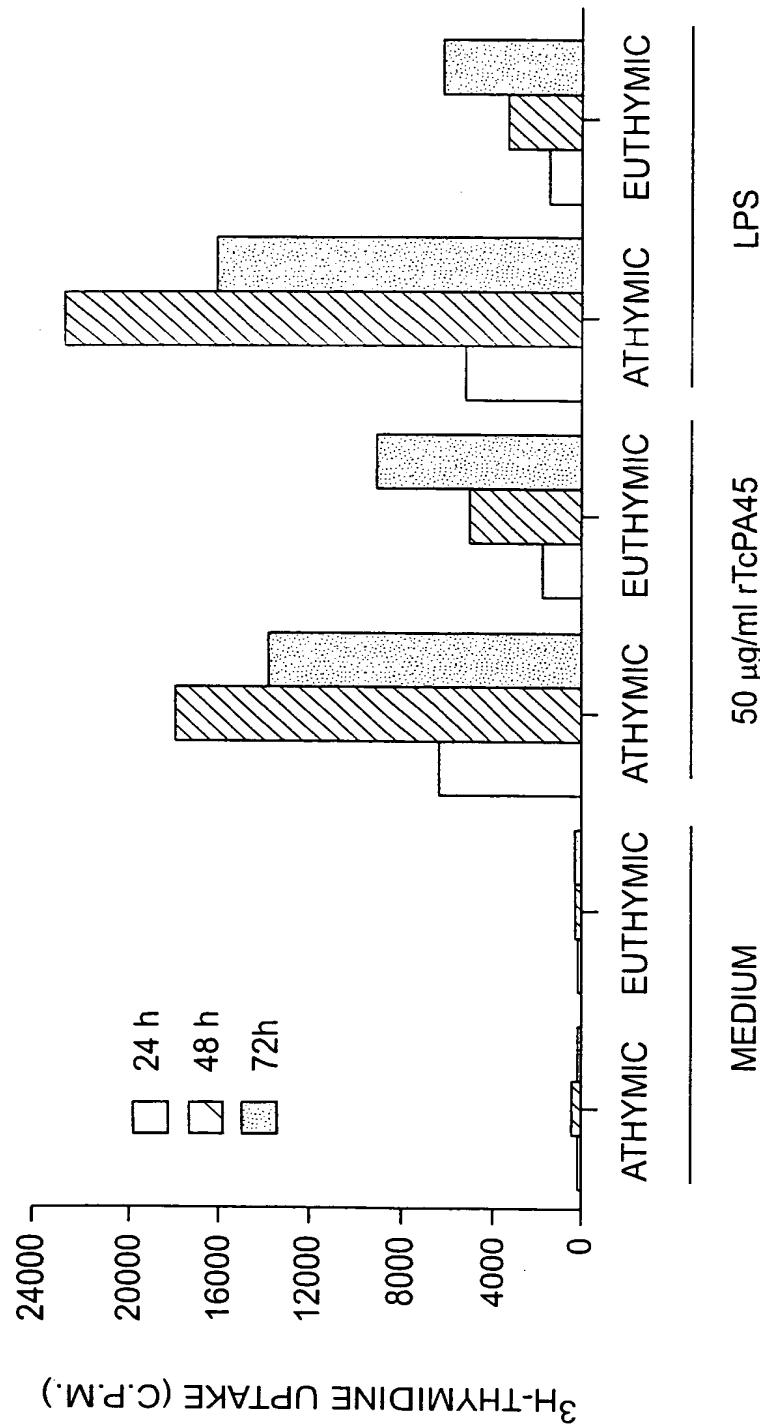
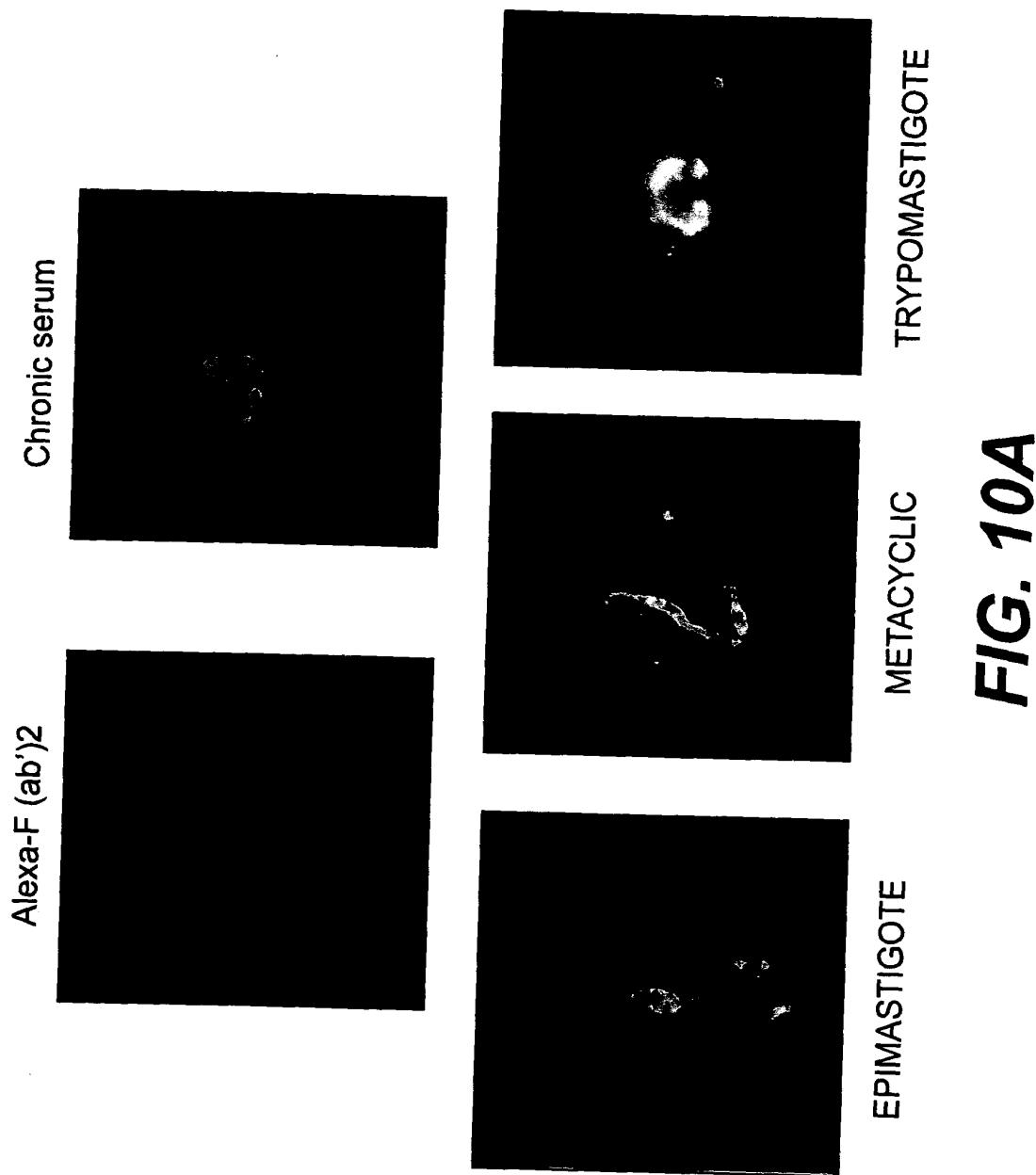


FIG. 9



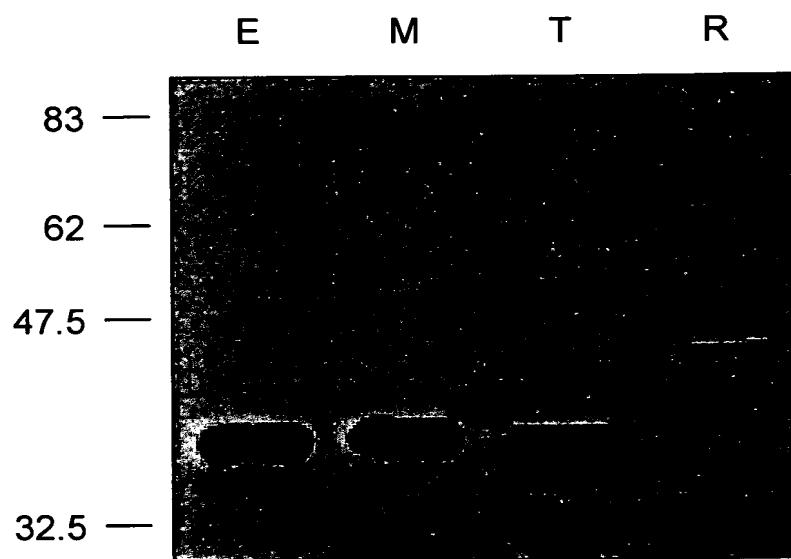


FIG. 10B

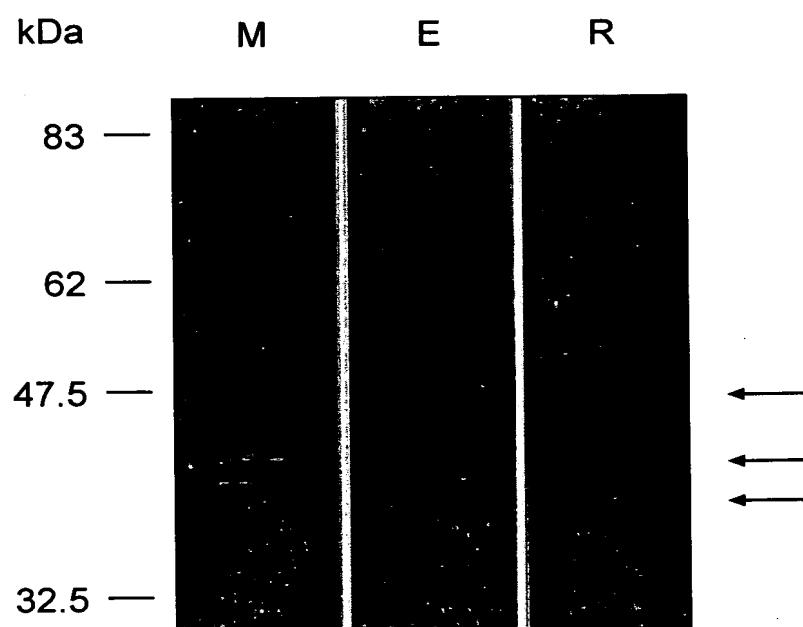


FIG. 10C

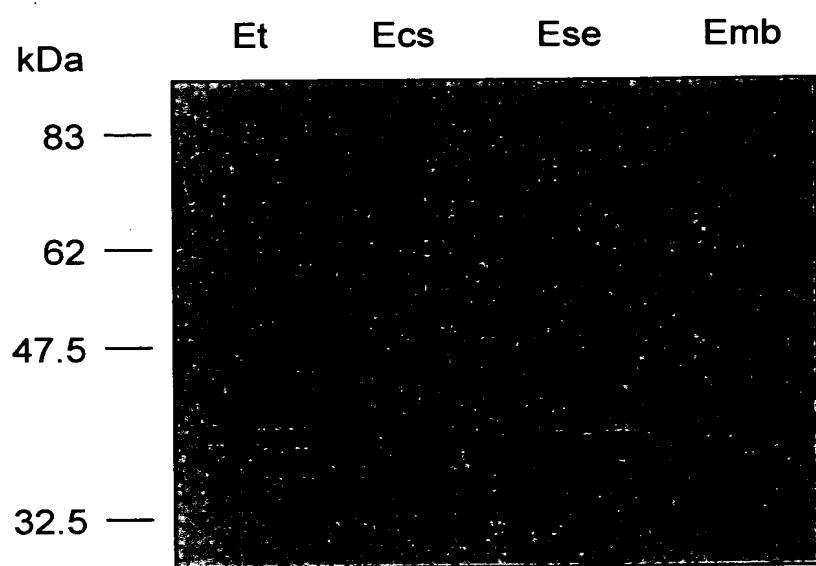


FIG. 10D

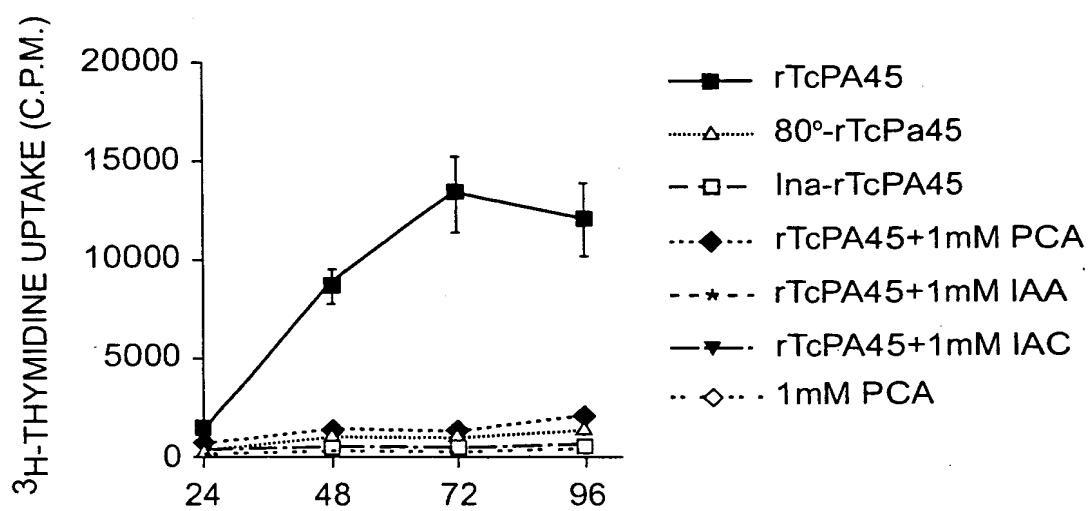


FIG. 11A

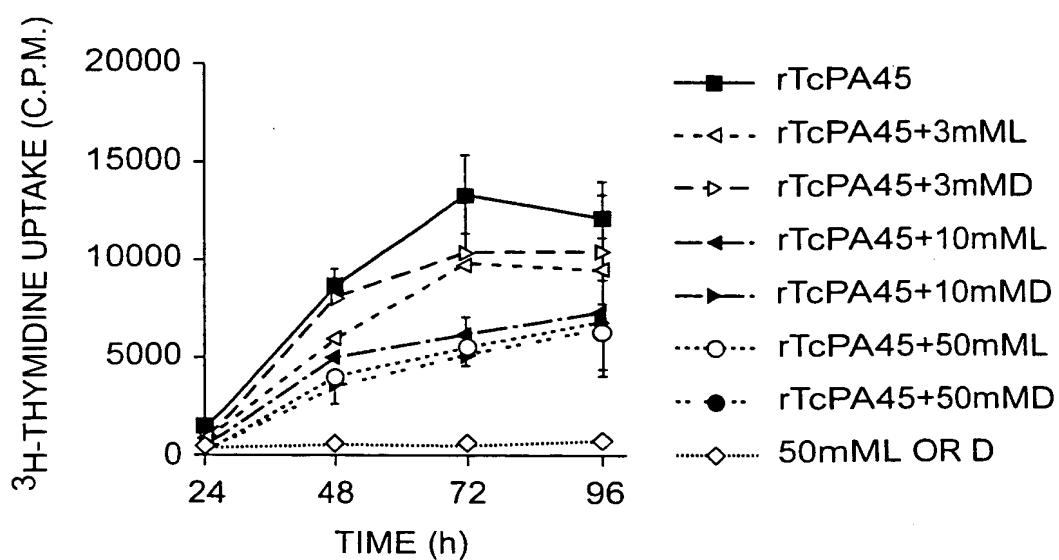


FIG. 11B

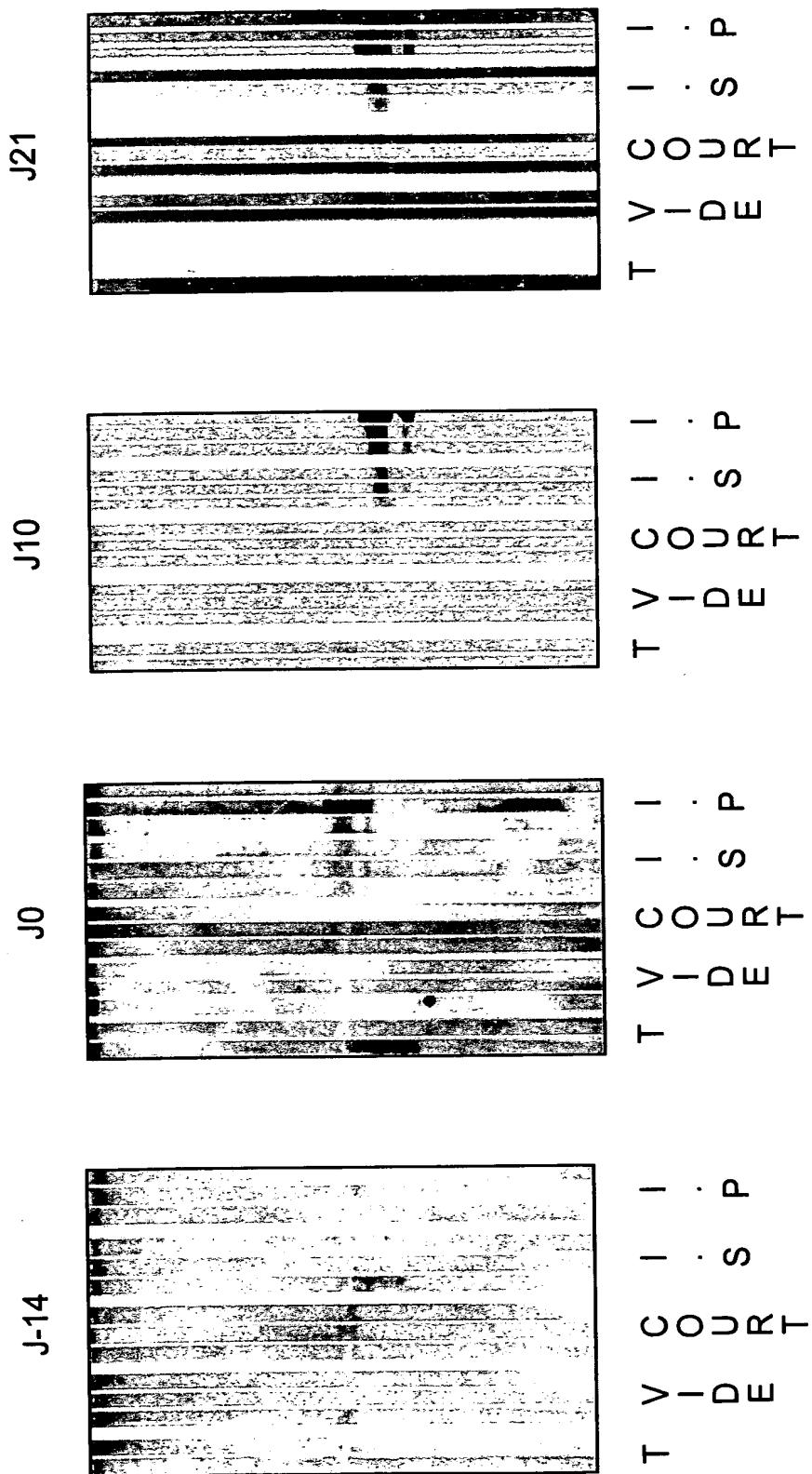


FIG. 12

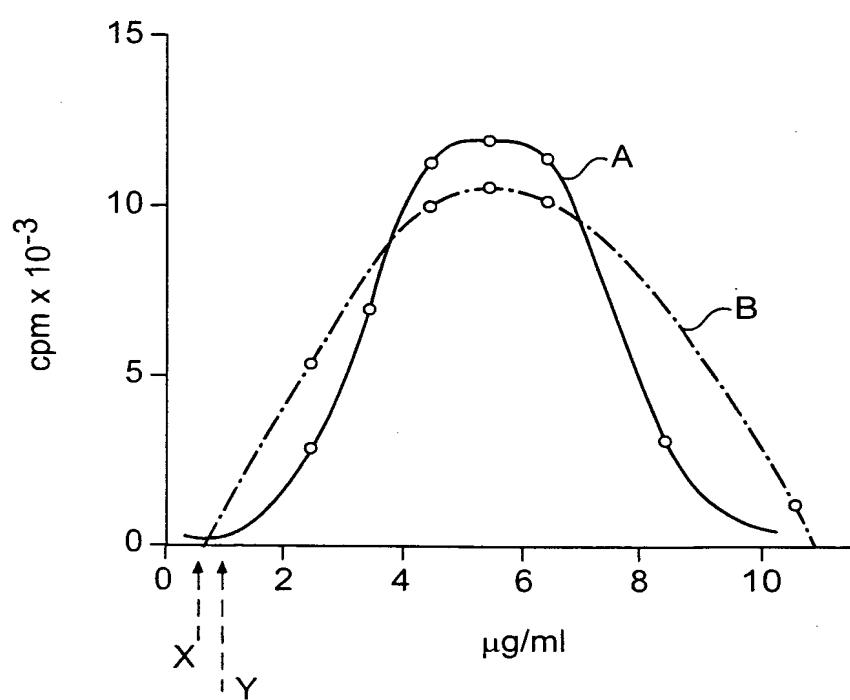
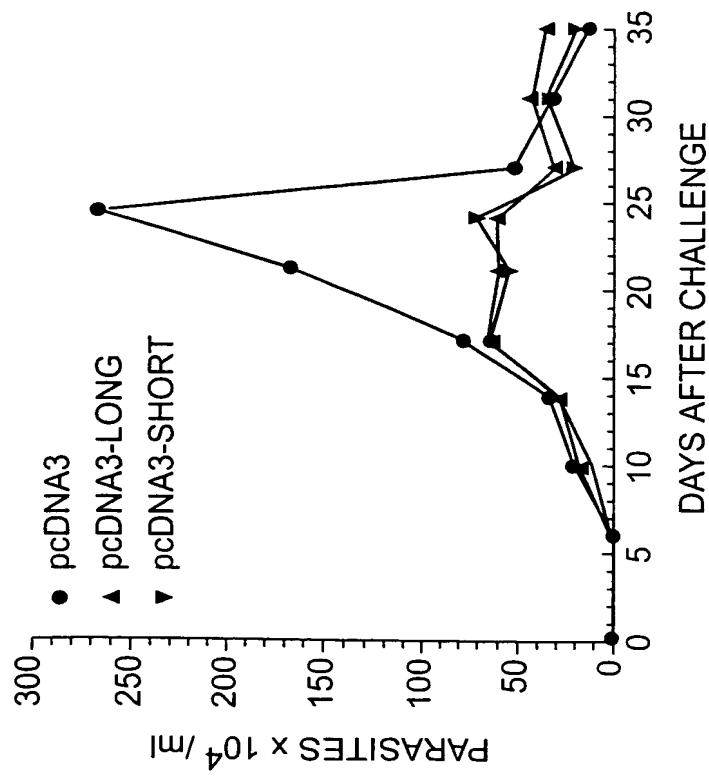


FIG. 13

DNA VACCINATION



rTcPA45 SUB-MITOGENIC INJECTION

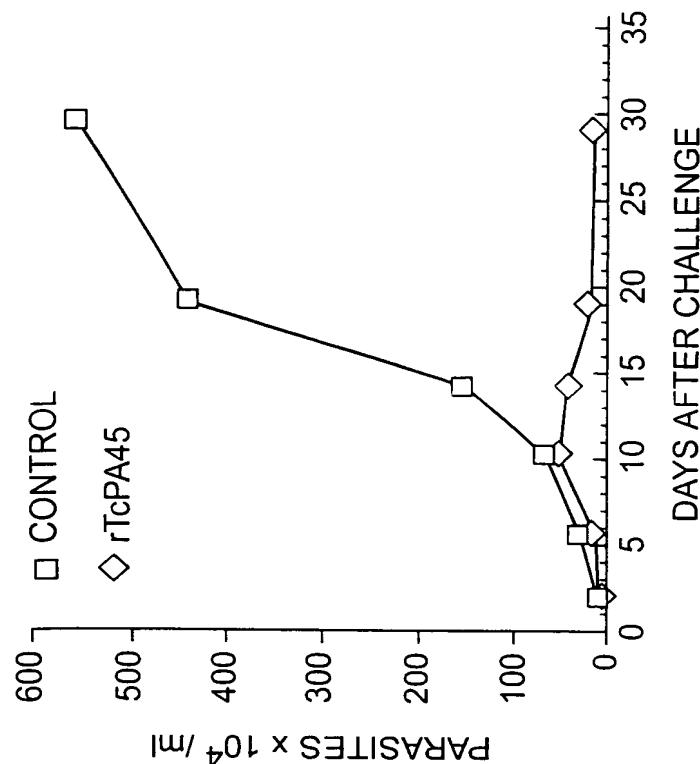


FIG. 14A

FIG. 14B

SEQ ID NO:2

TC	RTGQEKLLE <u>DQKYK</u> <u>LI</u> GEKKEKKNQRANRREHQQQKREIMRFKKS	75
TC	FTCIDMHTGEAARI <u>VTSGLPHIPGSNMAEK</u> KAYLQENMDYLRRGIMLEPRGHDDMFGAFLFDPIEEGADLGMVF	150
TC	MDTGGYLNMCGHNSIAAVTAAVET <u>TV</u> PAKAT <u>TV</u> PAKAT <u>TV</u> VVLDTPAGI <u>VRGTAH</u> IQS <u>GT</u> SEVSNASIIINVPSFLYQ	225
TC	QDVVVVLPKPYGEVR <u>VDIAFGGNFF</u> AI <u>VP</u> AE <u>QLGIDISVQNL</u> SRLQEAGELLRTEINRSVKVQHPQLPHINTVDC	300
TC	VEIYGPPTNPEANY <u>KNNVVF</u> FGN <u>RQADR</u> SPCGT GTS <u>AKM</u> ATLYAK <u>GQLR</u> IGETFVYESILGSLFQGRV--LGEE	371
TC	RIPGVKVPTKDAE <u>EGMLVV</u> TAEITGKAFIMGENTMLFDPTDPFKNGFTLKQ*	423

FIG. 15

SEQ ID NO:4

TC	FTCIDMHTEGEAARI <u>VTSGLPHIPGSNMAEK</u> KAYLQENMDYLRRGIMLEPRGHDDMFGAFLFDPIEEGADLGMVF	75
TC	MDTGGYLNMCGHNSIAAVTAAVETGIVSVPAKAT <u>NVPV</u> LDTPAGI <u>VRGTAHILQSGTESEVSNASII</u> INVPSFLYQ	150
TC	QDVVVVLPKPYGEVR <u>VDIAFGGNFFAIVPAEQLGIDISVQNL</u> SRLOEAGELLRTEINRSVKVQHPQLPHINTVDC	225
TC	VEIYGPPTNPEANYK <u>NVVIFGMRQADR</u> SPCGT GTSAKMATLYAKGQLRIGETFVYESILGSLEQGRV--LGEE	300
TC	RIPGVKVPVTKDAEEGMLVVTAEITGKAFIMGENTMLFDPTDPFKNGFTLKQ*	371
TC		423

FIG. 16

SEQ ID NO:7

POLYPYRIMIDINE RICH REGION



**SPLICE LEADER
ACCEPTOR SITES**

SIGNAL PEPTIDE

CCTTTTCTTTAAAAACAAAAATTCCGGGGAAATATGGAACAGGGTATATCGTAAAGTGTCTGTC
TTTCCGCCTCCATTTTTTGTGTGTTCCCTGATCTCGAACAGGGCAGGAAAGCTTCTGTTGACCAAAATAT
F S A F P F F C V F P L I S R T G Q E K L L F D Q K Y 42
AAAATTAAAGGGAGAAAAAGAAAAAGAAAAAAATCAACAGGAAACAGGAGAGAACACCAACAAAAAGGGAAATTATGCGATT
K I I K G E K K N Q R A N R R E K Q Q K R E I M R F 72
AAGAAATCATTACATGGCATCGACATGCATACGGAAAGGTGAAGCAGGACGGATTGTGACGAGTGGTTGCACACATCCAGGTTCGAAT
K K S F T C I D M H T E G E A A R I V T S G L P H I P G S N 102
ATGGGGAGAAAGCATACCTGCAGGAAACATGGATTATTGAGGGCATTAATGCTGGAACCACGTGGTCATGATGATATGTT
M A E K K A Y L Q E N M D Y I R R G I M L E P R G K D D M F 132
GGAGGCCTTTTATTGACCCATTGAAAGGGCCTGACTTGGCATGGTATTATGGATAACGGTGGCTATTAAATATGTTGGACAT
G A F L F D P I E E G A D L G M V F M D T G G Y L N M C G H 162
AACTCAATTGAGCGGGTTACGGGGCAGTTAACGGGAATTGGAGCGTGGCGGAAAGGCAACAAATGGTCCGGTGTCTGGACACA
N S I A A V T A A V E T G I V S V P A K A T N V P V V L D T 192
CCTGGGGTTGGTGGCGGGTACGGCACACCTTCAGAGTGGTACTGAGAGTGGTCAAATGGCAGTTTATCAATGTACCCCTCATTT
P A G L V R G T A R L Q S G T E S E V S N A S I I N V P S F 222
TTGTATCAGCAGGATGGTGGTGGTGTGGTGTGGTGAAGTACGGCCTATGGTGAATTGCAATTGGAGGCAATTGGCCATT
L Y Q D V V V L P K P Y G E V D I A F G G N E F A I 252

FIG. 17A

GTTCCCGGAGCAGTTGGATTGATATCTCCGTCAAAACCTCTCCAGGCTGGAGGAGAACTTCTGGGTACTGGAAATCAAT 880
 V P A E Q L G I D I S V Q N L S R L Q E A G E L L R T E I N 282
 CGCAGTGTGAAGGTTCAAGCACCCTCAGCTGCCATTAAACACTGTGGACTGTGGATATACTGGTCCGCCAACGAACCCGGAGGCA 970
 R S V K V Q H P Q L P H I N T V D C V E I Y G P P T N P E A 312
 AACTACAAGAACGTTGATATTGGCATATGGTGTGATCTCCATGGGACAGGCACCGGCCAACAGTGGCAACACTTTAT 1060
 N Y K N V V I F G N R Q A D R S P C G T G T S A K M A T L Y 342
 GCCAAAGGCCAGCTTCGCATCGGAGAGACTTTGTGTACGAGAGCATACTCTGGCTCACTCTCCAGGGCAGGGTACTTGGGAGGCGA 1150
 A K G Q L R I G E T F V Y E S I L G S L F Q G R V L G E E R 372
 ATACCGGGGTGAAGGTGCCGGTGAACAAAGATGCCGAGGAAGGGATGCTCGTTAACGGCAGAAATTACTGGAAAGGCTTTATCATG 1240
 I P G V K V P V T K D A E E G M L V V T A E I T G K A F I M 402
 GGTTCACACCATGCTGTGTTGACCCAACGGATCCGGTTAACGAACGGATTACATAAACGAGTACATGGTAGACGCCAACAACTATT 1330
 G F N T M L F D P T D P F K N G F T L K Q * 423
 GGGGAACACGTGCGAACAGGTGCTACGTGAAGGGTATTGAATCGTTTATTAGTGCATT 1420

ATTATAAATTTCATCTTACCCAAACCCCCCTTGGTTTCAACGGTACCCGTTGGGAGGAAAGCGATAAGGGGGACAAATTGGCTTTAT 1510
TTTCGATTTCATCTTACCCAAACCCCCCTTGGTTTCAACGGTACCCGTTGGGAGGAAAGCGATAAGGGGGACAAATTGGCTTTAT 1600

AGGAATAAACATATTCAATTTCATATCTTGGAAATCAAAAGGCAT
1651

bs: UNDERLINED THE SEQUENCED PEPTID
FOR CLONING

(b) NUCLEOTIDE SEQUENCE AND PEPTIDE SEQUENCE TcPA45

FIG. 17B

Obs: UNDERLINED THE SEQUENCED PEPTIDES USED TO DEDUCE DEGENERATED PRIMERS
FOB CLONING

SEQ ID NO:8

<u>TTTCCGCCTCCATT</u>	<u>ATGGCGTAAAGTGTCTGCCCCAACAAAAATT</u>	90
<u>F S A F P F F F</u>	<u>M R K S V C P K Q K F F</u>	12
<u>V F C V F P L I S R T G Q E K L F D Q K Y</u>	<u>180</u>	
<u>AAAATTATTAAAGGGCGAGAAAAGAAAAAGAAAAATCAACGGAGCAAACAGGAGAGAACACAAACAAAAAGGGAAATTATGGGATT</u>	<u>42</u>	
<u>K I I K G E K K E K N Q R A N R R E H Q Q K R E I M R F</u>	<u>270</u>	
<u>AGAAATCATTACATGGCATCGACATGGCATACGGAAAGGTGAAGCAGGATTGTGACGGAGTGGTTGCCACACATTCCAGGTTGGAAT</u>	<u>72</u>	
<u>K K S F T C I D M H T E G E A A R I V T S G L P H I P G S N</u>	<u>360</u>	
<u>ATGGCGGAGAAGGATACCTGCAGGAACATGGATTATTGAGGCCATGGCTGGCATGGAAACCACGTGGTCATGATGATGTT</u>	<u>102</u>	
<u>M A E K K A Y L Q E N M D Y L R R G I M L E P R G H D D M F</u>	<u>430</u>	
<u>GGAGCCTTTTATTGACCCATTGAGAAAGGGCTGACTTGGGATGGTATTCAATGGATACCGGTGGCTATTAAATATGTGGACAT</u>	<u>132</u>	
<u>G A F L F D P I E E G A D L G M V F M D T G G Y L N M C G H</u>	<u>520</u>	
<u>AACTCAATTGGCAGGGTTACGGGGCAGTTGAAACGGGAATTGTGAGGGTGGTACACTGAGAGTGGTCAATGGGAGTATTATCAATGTACCCCTCATTT</u>	<u>162</u>	
<u>N S I A A V T A A V E T G I V S V P A K A T N V P V V L D T</u>	<u>610</u>	
<u>CTCTGGGGTTGGTGGCGGGTACGGCACACCTTCAGAGTGGTACTGAGAGTGGTCAATGGGAGTATTATCAATGTACCCCTCATTT</u>	<u>192</u>	
<u>P A G L V R G T A H L Q S G T E S E V S N A S I I N V P S F</u>	<u>700</u>	
<u>TTGTATCAGGCAGGATGGTGGGGTGTGGCAAAAGCCCTATGGTAAGGTACGGGTGATATTGCATTGGAGGAATTTCGCCATT</u>	<u>222</u>	
<u>L Y Q Q D V V L P K P Y G E V R V D I A F G G N F F A I</u>	<u>790</u>	
<u>GTTCCCGGGAGCAGTTGGGAATTGATATCTCCGTTCAAAACCTCTCCAGGCTGCAGGAGGCAGGAACCTCTGGCTACTGAAATCAAT</u>	<u>252</u>	
<u>V P A E Q L G I D I S V Q N L S R L Q E A G E L L R T E I N</u>	<u>880</u>	
<u>CGCAGTGTGAAGGTTAGGACCCCTCAGCTGCCCATATAACACTGTGGACTGTGTGAGATATAACGTGGTCCGCCAACGAACCCGGAGGCA</u>	<u>282</u>	
<u>R S V K V Q H P Q L P H I N T V D C V E I Y G P T N P E A</u>	<u>970</u>	
		<u>312</u>

FIG. 18A

AACTACAAGAACGTTGTGATATTGGCAATCGCCAGGGGATCGGCTCTCCATGTGGCACAGGCCACCCAGGCCAAGATGGCAACACTTTAT 1060
N Y K N V V I E G N R Q A D R S P C G T G T S A K M A T L Y 342
GCCAAAGGCCAGCTTCCGATCGGAGAGACTTTGTGTACCGAGAGCATACTCGGCTCACTCTCCAGGGCAGGGTACTTGGGAGGAGCGA 1150
A K G Q L R I G E T F V Y E S I I G S L F Q G R V L G E E R 372
ATACCGGGGGTGAAGGTGCCGGTGAACAAAGATGCCGAGGAAGGGATGCTCGTTGTAACGGCAGAAATTACTGGAAAGGCTTTATCATG 1240
I P G V K V P V T K D A E E G M L V V T A E I T G K A F I M 402
GTTTCAACACCATGCTGTTGACCCCAACGGGATCCGTTAACGAAACGGATTACATTAAGCAGTAGATCTGGTAGAGCACAGAAACTATT 1330
G F N T M L F D P T D P F K N G F T L K Q * 423
GGGAACACCGTGCAGAACAGGTGCTACGTGAAGGGTATTGAATGAATCTGGTTTATTATTATTATTAGTCATT 1420

ATTATAAATTTTTTTGTGTTCAACGGTACCGGTGGGAGGGAAAGCGATAAGGGCGGACAATTGGCTTTGCTTTAT 1510

TTTCATTTCATCTTCTACCCAACCCCTTGGTTCCACCGGTGGGGTCTTGTGGTGGAGGAGCTTAATCCGCACCTCGG 1600

AGGAATAAACATATTCAATTTCATATCTTGAATCAAAAGGCAAT 1651

POLYADENILATION SITE

Obs: UNDERLINED THE SEQUENCED PEPTIDES USED TO DEDUCE DEGENERATED PRIMERS
FOR CLONING

NUCLEOTIDE SEQUENCE AND PEPTIDE SEQUENCE TcPA45

FIG. 18B

SEQ ID NO:9

FIG. 19A

AGGAATAAACATATTCAATTCTATCTTGGAAATCAAAGGCAT

POLYADENYLATION SITE

Obs: UNDERLINED THE SEQUENCED PEPTIDES USED TO DEDUCE DEGENERATED PRIMERS FOR CLONING

NUCLEOTIDE SEQUENCE AND PEPTIDE SEQUENCE TCPA45

FIG. 19B

SEQ ID NO:10

SIGNAL PEPTIDE

ATGCGTAAAGTGTCTGTCCCACAAAAATT

TTTTCCGCCTTCCCATTTTTTTTTGTGTGTTCCCTTGATCTCT

NUCLEOTIDE SEQUENCE OF SIGNAL SEQUENCE TcPA45

FIG. 20

SEQ ID NO:11

AAGAAATCATTCACATGCCATCGACATGCCATACGGAAAGGTGAAGCAGGATTGTGACGAGTGGTTGCCCCACACATCCAGGTGCAAT
K K S F T C I D M H T E G E A A R I V T S G L P H I P G S N 132
ATGGCGGAGAAAGCATACCTGCCAGGAAACATGGATTATTGAGGGCATTAATGCTGGAACCCACGTGGTCAATGATGATATGTTT 520
M A E K K A Y L Q E N M D Y L R R G I M L E P R G H D D M F 162
GGAGCCTTTTATTGACCCTATTGAAGAAGGGCTGACTTGGCATGGTATTCAAGGATAACGGTGGCTATTAAATATGTGGACAT 610
G A F L F D P I E E G A D L G M V F M D T G G Y L N M C G H 192
AACTCAATTGCGAGCGGTTACGGCGCAGTGTGAAACGGGAATTGTGAGCGTGGCGTGGCGAAGGCAACAAATGTTCCGGTTGTGGACACA 700
N S I A V T A A V E T G I V S V P A K A T N V P V L D T 222
CCTGGGGTTGGTGGCGGTACGGCACACCTTCAGAGTGGTACTGAGAGTGAGGTGCAATGGAGTTTCAATGTACCCCTCATTT 790
P A G L V R G T A H L Q S G T E S E V S N A S I I N V P S F 252
TTGTATCAGCAGGATGTGGTGGTTGCTGAAAGCCCTATGGTGAAGTACGGGTGATATTGCAATTGGCAATTTTTTCGCCATT 880
L Y Q D V V V L P K P Y G E V R V D I A F G G N F A I 282
GTTCCCGGGAGGAGTGGAAATTGATATCTCCGTTCAAAACCTCTCCAGGCTGCAGGAGGAACCTCTGGTACTGAAATCAAT 970
V P A E Q L G I D I S V Q N L S R L Q E A G E L L R T E I N 312
CGCAGTGTGAAGGTTAGCCCTCAGCTGCCCATTTAACACTGTGGACTGTGTGAGATATACGGTCCGCCAACGAACCCGGAGCA 1060
R S V K V Q H P Q L P H I N T V D C V E I Y G P P T N P E A 342
AACTACAAGAACGTTGTGATATTGGCAATCGCCAGGGATCGCTCTCCATGTGGACAGGGCACAGGCCAACACTTAT 1150
N Y K N V V I F G N R Q A D R S P C G T G T S A K M A T L Y 372
GCCAAAGGCCAGCTTGGCATCGGAGGACTTTGTGACGAGGATACTCGGCTCACTTCAGGGCAGGGTACTTGGGAGGGAGCGA 1240
A K G Q L R I G E T F V Y E S I L G S L F Q G R V L G E E R 402

FIG. 21A

ATACCCGGGGTGAAGGTGCCGGTACCCAAAGATGCCGAGGAAGGGATGCTCGTTGTAACGGCAGAAATTACTGGAAAGGCTTTATCATG 1330
I P G V K V P V T K D A E E G M L V V T A E I T G K A F I M 423
GGTTCAACACCATGCTGTTGACCCAACGGATCCGGTTAACAGAACGGATTACACATTAAGCAGTAGATCTGGTAGAGGACACAGAACTATT 1420
G F N T M L F D P T D P F K N G F T L K Q *
GGGAACACGTGCGAACAGGTACCGTGAAGGGTATTGAATGAATCGTTTATTATTATTAGTGCATT 1510
ATTATAAATTTTTTTGTTGGGTTCAACGGTACCGGTTGGGAGCAGGAAGCGATAAGGGGACAATTGGCTTTAT 1600
TTTCATTTCATCTTCTACCCAAACCCCTTGGTTCACCGGTCGGGGCTTGTGGTGGAGGTCTAAATCCGCACCTCGG 1651
AGGAATAAACATATTCAATTCATATCTTGAATCAAAGGCAT

FIG. 21B